Journal of Cleaner Production 211 (2019) 1412–1419

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Microalgae based biofertilizers: A biorefinery approach to phycoremediate wastewater and harvest biodiesel and manure

Shakeel A. Khan [a, *], Gulshan K. Sharma [a, b], Fayaz A. Malla [a, c, **], Amit Kumar [d], Rashmi [e], Navindu Gupta [a]

[a] Division of Environmental Sciences, Centre for Environmental Science and Climate Resilient Agriculture, ICAR- Indian Agricultural Research Institute, Pusa, New Delhi, 110012, India
[b] National Bureau of Soil Survey and Land Use Planning, Regional Centre Jorhat, Assam, India
[c] Environmental Sciences, Higher Education Department, Govt. of Jammu & Kashmir, Govt. Degree College Baghi Dilawar Khan Srinagar, 190002, India
[d] Department of Botany, Dayalbagh Education Institute, Agra, Uttar Pradesh, India
[e] CSIR-RAB, Library Avenue, Pusa Complex, New Delhi, India

ARTICLE INFO

Article history:
Received 26 June 2018
Received in revised form
20 November 2018
Accepted 29 November 2018
Available online 1 December 2018

Keywords:
Phycoremediation
Wastewater
Biorefinery
Chlorella
Biodiesel
Biofertilizers

ABSTRACT

The most important requirement of the agrarian advancement is resilient nutrient source for agriculture without jeopardizing the environmental assets and economy of the country. An algal biorefinery approach is the prime requirement for the sustainable production of biodiesel and biofertilizers after remediation of wastewater. In the present study, the microalgae *Chlorella minutissima, Scendesmus spp* and *Nostoc muscorum* and their consortium were used for the biorefinery approach. *C. minutissima* has shown maximum phycoremediation potential when compared to other possible microalga and their consortium. Experimental results showed that removal of NH_4^+-N, NO_3^--N, PO_4^{-3}-P, TDS, BOD_5 and COD were found to be 92, 87, 85, 96, 90 and 81% respectively. The maximum dry biomass was observed in *C. minutissima* followed by *Scendesmus spp*, and *N. muscorum*, i.e., $0.45 \pm 0.01\ g\,L^{-1}$, $0.44 \pm 0.02\ g\,L^{-1}$, $0.14 \pm 0.03\ g\,L^{-1}$ respectively. The nutrient fraction of nitrogen and phosphorus were maximum in *C. minutissima*, i.e., 5.46 ± 0.27 and 0.85 ± 0.03 respectively. The lipid productivity recorded maximum in *Scendesmus* ($81.23 \pm 4.5\ mg\,L^{-1}$) followed by *N. muscorum* ($14.29 \pm 8.7\ mg\,L^{-1}$) and *C. minutissima* ($11.33 \pm 5.6\ mg\,L^{-1}$). Using this biomass as manure one can save the chemical fertilizer of worth about 5584 $ (US Dollar) $ha^{-1}\ yr^{-1}$. The present study not only supports the sustainable phytoremediation, biodiesel production, and organic manure utilization directly but indirectly to combat climate change scenario through minimizing greenhouse gases production.

1. Introduction

Chemical fertilizer, fossil fuel and sewage wastewater are the three substantial issues across the world due to increasing population, urbanization, climate change and modern lifestyle (Singh et al., 2013; Fagodiya et al., 2017). These issues required an immediate sustainable solution for the betterment of nature and human life quality. A large volume of sewage wastewater is generated every day and globally >80% of all wastewater is discharged without treatment. Therefore, globally 20% of all wastewater is discharged with treatment. In which high-income, upper middle-income and lower middle-income countries contributes 38%, 28% and 8% respectively (WWDR, 2017). Urban India generates 61,948 million liter per day (MLD) out of which against 23,277 MLD are only treated. The treated wastewater in India is only 37% (MoEF&CC, GOI, 2018). The low-income (least developed) countries contribute the highest proportion of their used water due to lacking infrastructure, technical and institutional capacity, and financing. Globally, the reduction of untreated wastewater, recycling and safe reuse is highly required (Khan et al., 2018; Zhou et al., 2018a, 2018b). Apart from wastewater, firstly, the fossil fuel, are also

* Corresponding author. Center for Environmental Science and Climate Resilient Agriculture (CESCRA), NRL Building, Pusa Campus, Indian Agricultural Research Institute (IARI), New Delhi, 110012, India.,
** Corresponding author. Division of Environmental Sciences, Centre for Environmental Science and Climate Resilient Agriculture, ICAR- Indian Agricultural Research Institute, Pusa, New Delhi, 110012, India.
E-mail addresses: shakeel.iari@gmail.com, shakeel.khan@icar.gov.in, shakeel_iari@yahoo.com (S.A. Khan), fayaz@gdcbdk.ac.in (F.A. Malla).

https://doi.org/10.1016/j.jclepro.2018.11.281
0959-6526/

contributing to the climate change directly by emitting the carbon dioxide and indirectly spoiling the air quality through the generation of ozone and other secondary air pollutants (Pathak et al., 2016). Secondly, nitrogenous chemical fertilizer is highly used in the present day agriculture and directly contributing the climate change as nitrous oxide emitted from them and having high global warming potential among the greenhouse gases produced in agriculture management (Fagodiya et al., 2017). India is the third (5.6% of Global) biggest fossil fuel consuming country after China and USA. The crude oil (221.1 MTOE; 29.34%), natural gas (46.6 MTOE; 6.18%), coal (424 MTOE; 56.26%) are the important fossil fuel used in India (BP statistical Review, 2018). Globally, the fossil fuel consumption in 2016 was 132051.53 TWh (Terawatt-hours) (Smil, 2016). Biofuel production using microalgae grown in wastewater can also serve a cost-efficient and sustainable source of energy, nutrients and organic matter. Microalgae are having a higher photosynthetic efficiency (carbon fixation) than terrestrial plants in short duration and produce high biomass yield (Yu et al., 2018). Global biodiesel productivity of microalgae is estimated from 52000 to 121000 kg ha^{-1} yr^{-1} and in India it is 16.70–22.26 m^3 ha^{-1} yr^{-1} (Han et al., 2015). The potential benefits of microalgal biorefinery approach go well beyond human and environmental health (phycoremediation and GHGs mitigation), with implications on energy and food security as well as climate change mitigation.

However, currently available wastewater treatment techniques are effective but not sustainable sound due to their high cost, environment constraint and sludge generation. After conventional wastewater treatment processes, generated waste must be sent off-site for treatment and disposal, further increasing operational costs (Bosnic et al., 2000; Kabdasli et al., 2002). In comparison with physicochemical treatment technologies for wastewater, microalgae based treatments (phycoremediation) during the secondary treatment process of wastewater are more efficient and safe (Malla et al., 2015). Once the algal biomass generated, it can be used to produce biomanure, pharmaceuticals, biodiesel, ethanol, hydrogen, feed for fish and many other valuable products (Chisti, 2007; Khan et al., 2009; Sturm and Lamer, 2011). Various species of *Chlorella* and *Scenedesmus* could provide very high (>80%) and almost complete removal of ammonia, nitrate and total phosphorus from secondary treated wastewater (Martinez et al., 2000; Ruiz-Marin et al., 2010; Zhang et al., 2008). It has been reported by the researchers that microalgae have two significant advantages over higher plants concerning biomass generation (Sheehan et al., 1998). First, biomass yield was pointedly greater for microalgae; with productivities projected at about 70 metric tons per hectare per year (ha^{-1} yr^{-1}) of dry weight. Second, the cultivation of microalgae does not require arable land or fresh water. It can be carried out in wastewater. Nowadays, microalgae are used as feedstock for biofuel but apart from that harvested algal biomass can also be used as manure application for agriculture that too after harnessing the biofuel from the biomass (Fayza and Fattah, 2008). However, development of appropriate economically viable harvesting method of algae is a major challenge. It is well known and reported by many researchers that algae are one of the best media to remove excess nutrients such as nitrate, phosphate, ammonia, etc. from sewage wastewater (Chevalier et al., 2000). *C. minutissima* assimilate either ammonia or nitrate as N source in wastewater (Bhatnagar et al., 2010; Rawat et al., 2011). The harvested dried algal biomass can substitute commercial chemical fertilizers after harnessing the biofuel potential. Moreover, algal nitrogen would have much less potential for leaching or loss in run-off since only about 5% of the algal nitrogen would be available as mineral N at the time of application (Mulbry et al., 2005). Applying dried algal biomass to soils would not result in NH_3 volatilization as in case of other manures (Thompson and Meisinger, 2002). Nitrogenous fertilizers consumption in agriculture as nutrient was 110.18 and 16.73 million tonne (Mt) globally and in India respectively (FAO, 2018).

In the present study, one can estimate the phycoremediation, biomass production and manure synergistic potential of the three algae and their consortium of harvested algal biomass after lipids extraction. Further studies are needed to optimize the process at pilot scale and to assess the long-term manurial potential in different crops in field condition.

2. Material and methods

2.1. Study area

A network of sewage drains criss-crosses the Indian Agricultural Research Institute (IARI) campus having discharge amounts of about 20 Million Liters per Day (MLD). The wastewater entering in this conduit is treated only for primary treatment and intermittently applied for irrigation to crop fields.

2.2. Microalgae and growth condition

Microalgal strains *C. minutissima, N. muscorum* and *Scendesmus spp* were obtained and cultured at National Centre for Cultivation and Utilization of Blue Green Algae, IARI, New Delhi, India. The *C. minutissima* and *Scendesmus spp* were cultivated in Bold's Basal Medium (Stein, 1973), while *N. muscorum* was grown in BG-11 media (Allen and Steiner, 1968).

2.3. Wastewater collection and characterization

The samples of wastewater were collected in 20 L capacity of well-marked plastic bottles during August in the forenoon from IARI (primary treated). Before collecting, it was washed with non-phosphate detergent (NPD) and finally rinsed with distilled de-ionized water (DDW). Raw wastewater samples were characterized by various parameters viz., pH, total dissolved solids (TDS), electrical conductivity (ECw), dissolved oxygen (DO) Winkler method, nitrate - nitrogen (NO_3^--N) UV Spectrophotometric Method, ammonium - nitrogen (NH_4^+-N) (Nesslerization), phosphorus (P) (Calorimetric), potassium (K) Flame Photometric Method, biological oxygen demand (BOD_5) (Titrimetric) and chemical oxygen demand (COD) oxidation method (APHA-AWA-WEF, 2005).

2.4. Biomass production and phycoremediation potential

The experiments were carried out in a plastic tray having a length of 45 cm and breadth of 30 cm in triplicate. The algal culture was centrifuged at 10,000 rpm for 5 min and collected (0.7 g L^{-1}) in 300 ml plastic tubes. Later on, this batch culture was inoculated in a plastic tray containing wastewater. The inoculated trays were kept in the open at ambient temperature and light. An average sunshine 15 and 12 h were given for growth during August and September. The average extra-terrestrial solar radiation (G0) was 30.78 and 20.64 MJ m^{-2}d-^{1} at the growth period. 5 ml sample from each tray were regularly withdrawn after every 48 h for the analysis of chlorophyll content. At the end of the experiment, homogenized broth from each tray was centrifuged at 10000 rpm for 5 min and the supernatant was collected for the analysis of pH, EC, TDS, DO, NO_3^--N, NH_3-N, P, K, BOD_5 and COD.

2.5. Biochemical composition and manure potential of microalgae

The biomass was harvested through flocculation using muslin

cloth followed by centrifugation. The harvested biomass dried at 60 °C for 24 h. Dried algal biomass was acid digested followed by analysis of NPK content through Kjeldahl, calorimetric and flame photometer methods.

2.6. Fatty acid profile and lipid extraction

The microalgae biomass was ground and dewatered for 20 min at 80 °C in an incubator. Ether was used for the separation of algal oil and kept it for settling for 24 h. The algal oil isolated by placing the flask on a continuous rotator shaker (200 RPM). Sodium hydroxide and methanol (mixture) were poured into the algal oil containing flask after removal of the solvent and kept it for 3 h on continuous rotator shaker to allow transesterification. The glycerin and pigment settled down at the bottom, and biodiesel was separated using separating funnel (Lepage and Roy, 1984). The lipids extraction has been done as per the modified method (Blight and Dyer, 1959). The gas chromatography technique with a flame ionization detector (FID) was used for the analysis of fatty acid methyl esters (FAMEs).

2.7. Reduction efficiency

The following equation calculated the reduction/removal efficiencies (%) of the various parameters (Ji, L et al., 2012).

$$R = \frac{(Ci - Ce)}{Ci} \times 100 \quad (1)$$

where

R = the removal/reduction percentage at each measurement time,
Ci = the initial concentration of a given parameter, and
Ce = the remainder concentration on a given sampling day

2.8. Statistical analysis

To evaluate the potential relationship between the different physico-chemicals parameters the Pearson correlation coefficient and calculation mean and critical difference (CD) between selected algae were made through IBM-Statistical Package of Social Sciences (SPSS 21).

3. Results and discussion

3.1. Phycoremediation

The initial characteristics of collected sewage wastewater and the residual concentration of the respective parameter after phycoremediation are summarized in Table 1. The maximum TDS and NO_3^--N removal (96% and 87%) were obtained with *C. minutissima*, while the maximum phosphorus removal (85%) was observed with the consortium. The maximum NH_4^+-N removal (92%) was observed with the *Scendesmus spp* (Fig. 1). However reduction in NH_4^+-N was nearly equal in all treatment, but the maximum reduction was found in *Scendesmus spp*. Usually, algae prefer to assimilate N in the form of ammonia because it is a passive way of assimilation and also reported that only 25–33% of the initial content was recycled in the protein biomass by cultures of *S. Obliqus* (Nunez et al., 2001). However, most of the NH_4^+-N part lost (presumably as ammonia gas to the atmosphere) as a result of the combined effect of the high pH values of the culture and the aeration. The amount of N recycled in the biomass was considerably higher with up to 80% of ammonia (Volensky, 2001; De Philippis et al., 2007). The discrepancy between the recycled N and the N removed may reasonably be ascribed to the loss of ammonia by degassing as well as to the adsorption properties of the cell walls of microalga. By comparing, it can be concluded that if NH_4^+-N and NO_3^-- N both were available, microalgae preferred to utilize NH_4^+-N first. The previous studies have also reported the same result (Li et al., 2010; Perez-Garcia et al., 2011; Su et al., 2012; Nathan et al., 2016). Maximum and minimum nitrate reductions were found in case of *C. minutissima* (87%) and *N. muscorum* (20%). It may be due to a minimum reduction of nitrate in case of *N. muscorum* might be due to nitrification process as *Nostoc* is a nitrogen fixer, it requires a less external source of nitrogen for its growth and development. Hence, nitrogen is an essential nutrient for the production of microalgae biomass.

TDS decreased sharply up to 10 days, after that it declined at a slow pace in a 25 days cycle. It was detected that maximum TDS reduction found in case *C. minutissima* followed by the consortium. The decrease in TDS was due to ingestion of dissolved solids from wastewater which is nutrient rich for the growth of microalgae. EC depends on the overall ionic concentration in water; it has often been used as an index of the TDS in water. Pure water is a weak

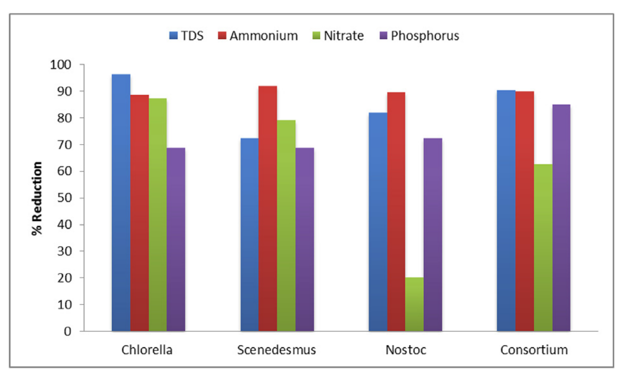

Fig. 1. Percent reduction of TDS, phosphorus and nitrogen as NH_4-N and NO_3-N.

Table 1
Physico-chemical composition of sewage wastewater after phycoremediation.

Parameter	Initial value	*C. minutissima*	*Scenedesmus spp*	*N. muscorum*	Consortium	C.D.
pH	8.01	8.82 ± 0.05	9.09 ± 0.09	9.32 ± 0.05	9.21 ± 0.02	0.09
EC (dS/m)	3.14	0.12 ± 0.03	0.98 ± 0.01	0.59 ± 0.01	0.10 ± 0.02	0.09
TDS (mg/l)	2196	81.73 ± 17.59	606 ± 8.32	396.6 ± 10.86	212.73 ± 8.88	39.73
P (ppm)	3.68	1.15 ± 0.08	1.15 ± 0.03	1.02 ± 0.02	0.55 ± 0.03	0.16
K (ppm)	15.65	14.58 ± 0.60	14.63 ± 0.49	14.95 ± 0.27	13.75 ± 0.29	N/A
NH_4-N (ppm)	39.5	4.51 ± 1.17	3.13 ± 0.17	4.09 ± 0.03	3.99 ± 0.19	N/A
NO_3^-N (ppm)	2.38	0.30 ± 0.02	0.50 ± 0.01	1.90 ± 0.03	0.89 ± 0.01	0.07
DO (mg/l)	2.6	10.55 ± 0.03	10.16 ± 0.20	9.80 ± 0.17	10.96 ± 0.15	0.60
BOD_5 (mg/l)	99.5	3.36 ± 0.19	9.63 ± 0.08	9.89 ± 0.14	5.69 ± 0.12	0.70
COD	149.75	29.13 ± 0.01	30.13 ± 0.02	34.23 ± 0.03	28.02 ± 0.06	0.02

electrolyte, and the EC of aqueous solutions will thus depend on the presence of charged ions. Electrical conductivity increases with the number of ions in solution. Overall the change of EC in water can be related to the change in the electron mobility in water (the relation is inherently nonlinear because, at higher concentrations, interactions among ions can impede their mobility) and change in temperature (Moore et al., 2008).

Phosphorus extracted by algal cells mainly used for the production of phospholipids, adenosine triphosphate (ATP) and nucleic acids which get assimilated as inorganic orthophosphate, preferably as $H_2PO_4^-$ or HPO_4^{2-}. In contrast to nitrogen uptake the phosphorus uptake process is active, i.e., it requires energy. Maximum P reduction was observed in consortium followed by *N. muscorum*. Removal of P in case of *N. muscorum* was more in comparison to others because of more P require by *Nostoc* for nitrification. Similar results were also found in the previous studies where the assimilation was the primary P removal mechanism in phycoremediation (Su et al., 2011; Zhang et al., 2011). Almost every microalga and cyanobacteria require phosphorus for nitrification. An extended model based on the 'activated sludge have been used to investigate the kinetics of nitrification in the case of phosphorus deficiency, the demand for phosphorus under dynamic conditions, as well as the effect of variations in the nitrogen load at low levels of phosphate. The study revealed that the dominance of *Chroococcales* was coupled to low salinity and low total phosphorus, whereas *Oscillatoriales* correlated with high total nitrogen and low salinity. The increase *of Nostocales* in the coastal Bothnian sea was explained by a rise in total phosphorus and decrease in dissolved inorganic nitrogen compared to an increase of total nitrogen and phosphorus in the coastal Baltic proper (Andersson et al., 2015).

The pH value increases up to 10 days in all treatment after that it became constant. The reason for the increase in pH was due to photosynthesis by microalgae which reduces dissolved CO_2 concentrations and organic molecule. The inorganic chemicals usually used by microalgae are CO_2 and bicarbonate, the latter requiring the enzyme carbonic and hydrase to convert it to CO_2 (Borowitzka, 1998). The increase of pH in the medium was also reported by Tam and Wong (2000), which could lead to the precipitation of phosphorus and increasing phosphate adsorption on the surface of microalgae. The maximum pH increase has been found in the case of *N. muscorum* (pH 9.32) while in the event of *C. minutissima*, it was reached up to 8.8 (Fig. 2).

Dissolved oxygen (DO) of untreated wastewater varied from 1.8 mg L^{-1} to 4.3 mg L^{-1} with an average value of 2.6 ± 1.15 mg L^{-1}. After 25 days of algal cultivation, the DO content was increased from 2.6 ± 1.15 to 10.55 ± 0.03, 10.16 ± 0.20, 9.80 ± 0.17 and 10.96 ± 0.15 mg L^{-1} in the presence of *C. minutissima, Scendesmus spp, N. muscorum* and consortium, respectively (Table 1). Initially, up to 5 days of the cultivation, DO decreases because of high microbial activity and low algal growth in sewage wastewater (Fig. 2). But after 5th days it starts to increase until the last day, same has also been reported by Sengar et al. (2011). Results showed that *C. minutissima* was having the highest potential of BOD removal; hence increase in DO was ceiling after the phycoremediation. Least rate of increase of DO recorded in *N. muscorum* due to oxygen consumption by nitrifying bacteria and nitrification process. It has been observed that DO value for *Phormidium spp*. increased slower to the peak value. There is much reason for the increase in DO; it may be the absorption of oxygen from the atmosphere, reduction of nitrate and nitrite, photosynthetic oxygenation by algae. Out of above reason, photosynthesis might be the primary cause of the increase in DO in an aquatic habitat. Biological Oxygen Demand (BOD_5) of untreated wastewater varied from 78.1 mg L^{-1} to 125.2 mg L^{-1} with an average value of 99.5 ± 21.8 mg L^{-1}. After 25 days of algal cultivation, the BOD content was reduced from 99.5 ± 21.8 mg L^{-1} (Initial) to 3.36 ± 0.36, 9.63 ± 0.08, 9.89 ± 0.14 and 5.69 ± 0.12 mg L^{-1} in the presence of *C. minutissima, Scendesmus spp, N. muscorum* and consortium (Table 1). It has been observed that in all the treatments the removal of BOD was higher than 90% (Fig. 3). Considerable reduction in COD of wastewater was noted in all the culture after phycoremediation. Most top removal of COD was observed in case of consortium (81%) followed by *C. minutissima* (80%) (Fig. 3). When an organic carbon source is present in the medium and light is used for energy, algal growth is considered as photo heterotrophic growth. The photo heterotrophic growth of microalgae allows assimilating atmospheric CO_2 and organic carbon from aqueous medium simultaneously (Zhang et al., 2011).

Overall all the three species and their consortium depicted outstanding biomass productivity in IARI wastewater. However, phycoremediation potential of *C. minutissima* and *Scendesmus spp* was higher. Nevertheless, results indicate the suitability of the isolates to be grown on wastewater for mass cultivation as well as phycoremediation. The resultant biomass was further utilized for lipids and manure production.

3.2. Biomass production

Algal biomass was harvested on the 25th day of growth in the wastewater with the use of muslin cloth followed by centrifugation and was measured for dry weight. The maximum fresh and dry weight was observed in *Scendesmus spp.* and *C. minutissima* (Fig. 4). Reason for getting the maximum dry weight in *Scendesmus spp.* and *C. minutissima* were due to maximum growth regarding chlorophyll as shown in (Fig. 5). Prabakaran and Ravindran (2011) reported that

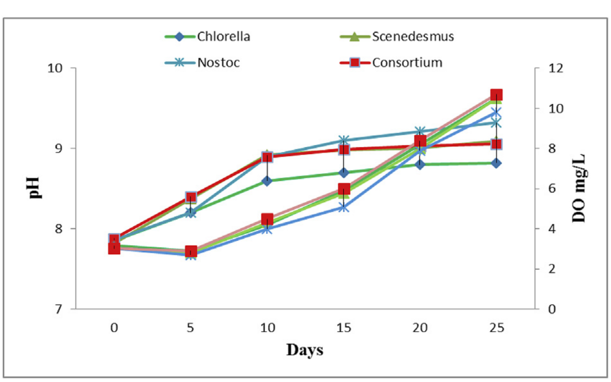

Fig. 2. Change in pH and DO during growth period.

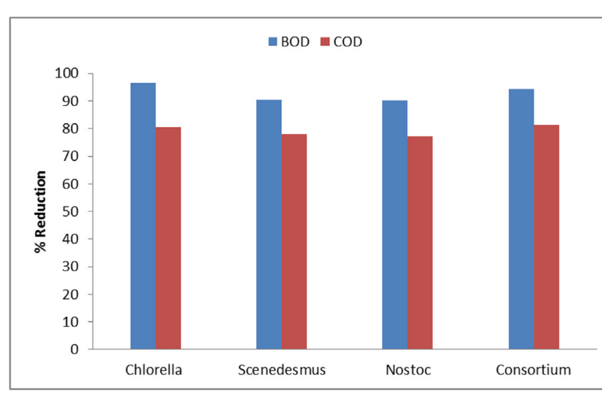

Fig. 3. Change in BOD and COD during growth period.

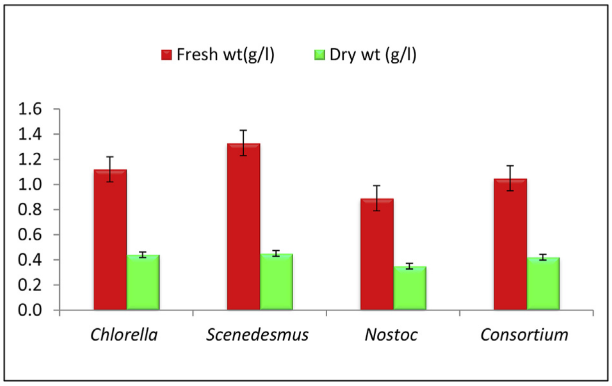

Fig. 4. Fresh and dry weight of algal biomass.

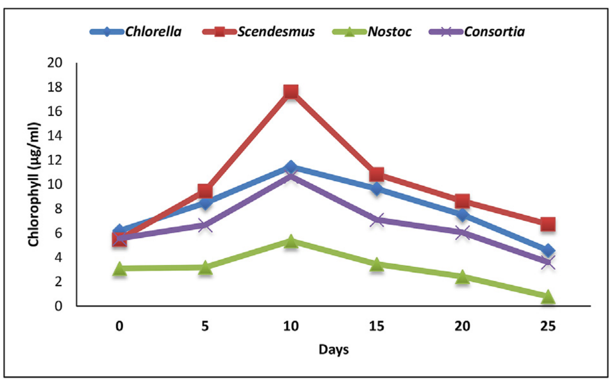

Fig. 5. Chlorophyll content during growth period.

after the 15th days of incubation, *Scenedesmus spp*. reached a growth rate of 0.38 ± 0.01 g L^{-1} higher than *Chlorella spp*. of 0.2 ± 0.01 g L^{-1}. The results of this study suggest that out of three microalgae, *C. minutissima* and *Scenedesmus spp*. could thrive best in wastewater and produce a factual amount of biomass after 20–25 days of incubation, in tandem remediate the wastewater. The consortium of three algae had not given the desired results and measured in lesser numbers comparatively (Fig. 5).

3.3. Fatty acid content, lipid content and lipid productivity

The fatty acid profile is one of the prospective indicators of biodiesel advantage. Usually, the C18 and C16 series substance of microalgae have been used to report the biodiesel production. The lipid productivity was recorded as 11.33 ± 5.6 mg L^{-1}, 81.23 ± 4.5 mg L^{-1} and 14.29 ± 8.7 mg L^{-1} in *C. minutissima, Scendesmus spp.* and *N. muscorum* respectively, which indicates *Scendesmus spp.*, has a better capacity for lipid production in wastewater as compared to other species. The average lipid content was recorded to be $19.23 \pm 13\%$, $15.65 \pm 55\%$ and $3.56 \pm 12\%$ for *C. minutissima, Scendesmus spp.* and *N. muscorum*, respectively, which shows that *C. minutissima* has higher lipid content. The fatty acid composition of *C. minutissima, Scendesmus spp.* and *Nostoc muscorum* was determined after 25 days and was found to be mainly composed of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). These acids account for 35.26/43.13/32.13%, 4.33/1.67/4.85%, 40.76/22.67/42.53%, 112.12/29.32/11.73%, 7.5/3.21/8.76% of total fatty acid for *C. minutissima,/Nostoc muscorum/Scendesmus spp*. (Fig. 6). The C18:0 and C18:3 in *N. muscorum* were found less as compared to

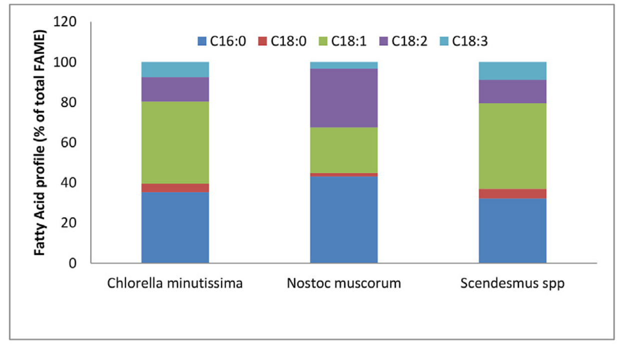

Fig. 6. Fatty acid composition.

C. minutissima and *Scendesmus spp.* which has also been reported by Kodandoor and Madaiah, 2011. The oleic acid content in *C. minutissima* and *Scendesmus spp*. was reported higher than *N. muscorum* which indicates the more top quality of biodiesel production (Cherng Yuan Lin and Lin Yi-Wei, 2012).

3.4. Nutrient potential of microalgae

The maximum manurial value regarding major nutrient (N) was found in *C. minutissima* i.e. $5.87 \pm 0.35\%$ as shown in (Fig. 7). Many researchers have reported the range of 3–7% nitrogen in *Chorella*. Wilkie and Mulbry (2002), have reported 4.9–7.1% N and 1.5–2.1% P in microalgae. Mulbry et al. (2007) reported 3.3–6.4% N in dry algal biomass. While Adey and Loveland (1998) reported 6–9% N and 1–2% P from harvested algal biomass.

3.5. Pearson correlation analysis

A Pearson correlation is given in (Table 2) between nutrient content of *C. minutissima* after phycoremediation and physico-chemical status of sewage wastewater. It was found that there was stronger associations correspond to phosphorus content of sewage wastewater and nutrient content of algae regarding NPK. It may be because phosphorus is an essential nutrient for growth and development of algae.

3.6. Feasibility analysis of phycoremediation of wastewater and manure production

The agriculture sector is devoted for nutrients such as N and P and it's required to enhance the productivity of land. India is the second largest consumer of fertilizers in the world after China, consuming about 38.6 Mt per year (FAI, 2015–16). Although India could manage its requirement from domestic industry, however,

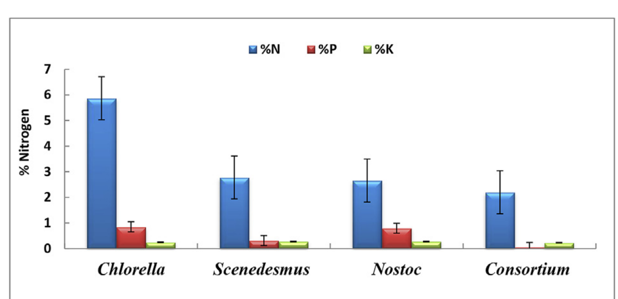

Fig. 7. Manure percentage in harvested algal biomass.

Table 2
Pearson Correlation coefficient between physico-chemical status of sewage wastewater and Algae Dry Biomass nutrient content (*Chlorella minutissima*).

Sewage Wastewater	Algae Dry Biomass nutrient content (*Chlorella minutissima*)		
Parameters	Nitrogen	Phosphorus	Potassium
pH	−0.241	−0.183	−0.320
EC	0.303	−0.113	−0.020
TDS	0.303	−0.163	−0.077
Phosphate	**0.635***	**0.570***	**0.601***
Potassium	0.259	0.317	0.384
Ammonia	0.276	−0.009	0.322
Nitrate	0.345	0.371	0.350
BOD_5	−0.227	−0.139	0.156

Correlation is significant at the 0.05 level (1-tailed).

during the last 7–8 years, there has been a significant increase in imports of N and P. Indian imports, which were about 2 Mt in the early part of 2000, rose to 13 Mt of fertilizers in 2011–12. On the other hand, the phycoremediation of sewage wastewater can possibly produce manure which would reduce import of fertilizers up to a certain extent.

It has been found in the experiment that the *C. minutissima* is having best biofertilizers potential along with the highest biomass and lipids production. Therefore, we have done the feasibility analysis of *C. minutissima* for manurial potential. After 25 days of culture, the dry weight in *C. minutissima* obtained was 0.44 kg (Fig. 4). Similarly, from this dry weight, 5.87% N, 1.15% P and 0.28% K were obtained (Fig. 7). Let's assume the experiment would be performed for one year and completion of every cycle of *C. minutissima* growth takes one month. Accordingly the feasibility integration of biomass from *C. minutisima* would be conducted in a pond having an area of 1 ha (10000 m^2), the total dry biomass obtained from *C. minutisima* would be 391111 kg ha^{-1} y^{-1}. Accordingly, from this dry weight, one could obtain N, P and K 22958, 4498 and 1095 kg ha^{-1} y^{-1} respectively (Fig. 8). Using this biomass in agriculture as biofertilizers, one can save the chemical fertilizers of about USD (United State Dollars) 55840 ha^{-1} y^{-1}. The associated negative impacts of using chemical fertilizers could be practically mitigated if one does the phycoremediation of wastewater by *C. minutisima* and additionally will get the biodiesel also. It was also assumed that harvesting of microalgae could make upto 20–30% of the total cost of production (Stein, 1973). So net profit would be approximately 4188 USD if one applies the dry algal biomass after harvesting as biofertilizers. Hence, the integration of algal biomass and lipids production vis a vis wastewater treatment by phycoremediation could be a viable proposition as energy-efficient techniques for the sustainable development and organic agriculture.

4. Conclusion

The results of this study suggest that growing algae in nutrient-rich sewage wastewater offer a novel prospect of applying algae to manage the pollution load of wastewater, generate biomass, extract lipids, and produce biofertilizers. Hence it serves the triple roles of pollution control, biofuel generation and submission of algal biomass as organic fertilizers. In addition to the potential

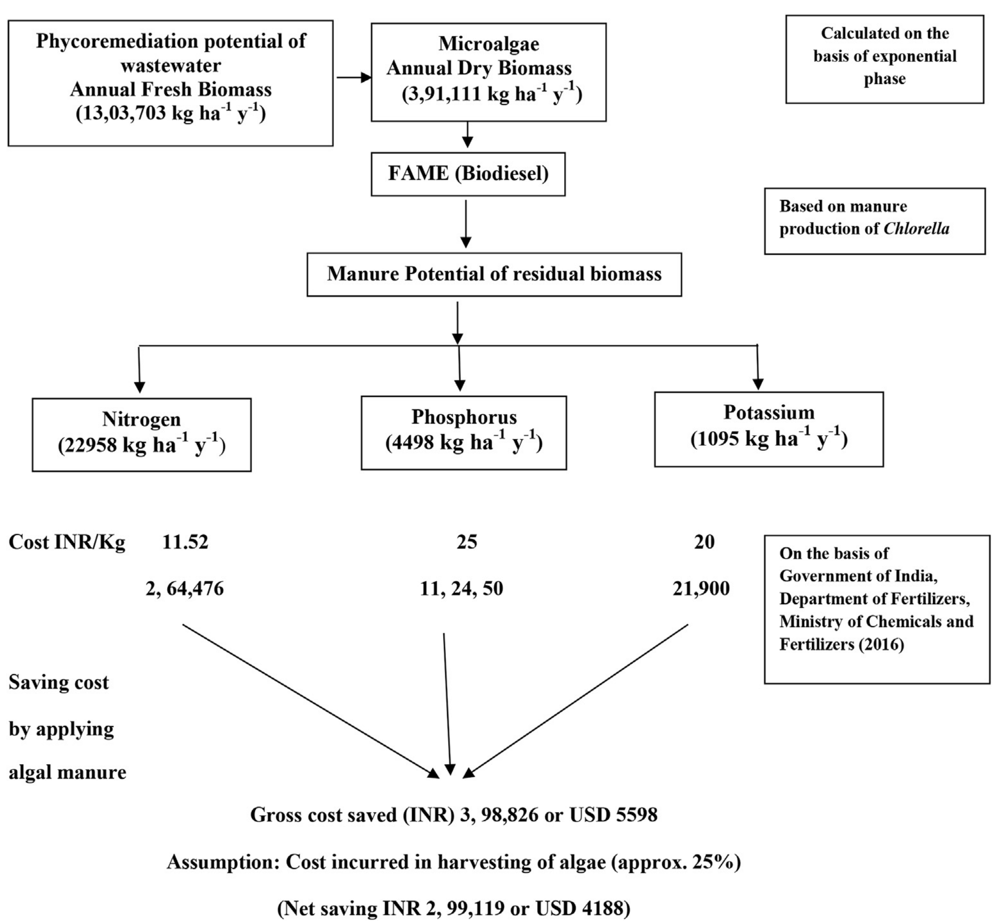

Fig. 8. Feasibility analysis of phycoremediation of wastewater and manure production.

predictability of algal biomass, it constitutes a stable, transportable and highly concentrated form of transformed manure nutrients. The algal biomass showed significantly high NPK content better than available organic fertilizers and relatively low nutrient content when compared to chemical fertilizers. As only about 5% of the algal N will be available as mineral N at the time of application in the field, hence the algal N would not a loss to leaching and surface runoff, thus reducing the eutrophication. This biofertilizers will enhance the soil micro and macro nutrients and enhance plant growth, for sustainable organic agriculture. This biorefinery approach with a zero-waste concept will boost the economy and environment of the country. The present study not only supports the sustainable phycoremediation, biodiesel production, and organic manure utilization directly but indirectly to combat climate change scenario through minimizing greenhouse gases. Further studies are needed to enhance the process at pilot scale and to assess the long-term manurial potential in different crops at the field level.

Acknowledgment

The authors sincerely acknowledge the laboratory facilities at National Centre for Cultivation and Utilization of Blue Green Algae, IARI, New Delhi, for their help during the culture experiments. This work was supported by the Indian Council of Agricultural Research - Indian Agricultural Research Institute and facilities provided by Centre for Environmental Science and Climate Resilient Agriculture, (CESCRA), IARI, New Delhi, India.

References

Adey, W.H., Loveland, K., 1998. In: Dynamic Aquaria: Building Living Ecosystems, second ed. Academic Press, San Diego, CA, p. 498.

Allen, M.M., Steiner, R.Y.J., 1968. Selective isolation of blue-green algae from water and soil. Gen Microbiol 51, 203. https://doi.org/10.1099/00221287-51-2-203.

Andersson, A., Hoglander, H., Karlsson, C., Huseby, S., 2015. Key role of phosphorus and nitrogen in regulating cyanobacterial community composition in the northern Baltic Sea. Estuar. Coast Shelf Sci. 164, 161–171. www.core.ac.uk/download/pdf/82127461.pdf.

APHA-AWA-WEF, 2005. In: Standard Methods for the Examination of Water and Wastewater, twenty-first ed. American Public Health Association, Washington DC.

Bhatnagar, A., Bhatnagar, M., Chinnasamy, S., Das, K., 2010. Chlorella minutissima – a promising fuel alga for cultivation in municipal wastewaters. Appl. Biochem. Biotechnol. 161, 523–536. https://doi.org/10.1007/s12010-009-8771-0. Epub 2009 Nov 1.

Blight, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 912–917. https://doi.org/10.1139/cjm-2017-0613.

Borowitzka, M.A., 1998. Limits to growth. In: Wong, Y.-S., Tam, N.F.Y. (Eds.), Wastewater Treatment with Algae. Springer-Verlag, New York, pp. 203–226.

Bosnic, M., Buljan, J., Daniels, R.P., 2000. Pollutants in Tannery Effluents. US/RAS/92/120, United Nations Industrial Development Organization, Regional Programme for Pollution Control in the Tanning Industry in South-east Asia.

BP Statistical Review of World Energy 2018. www.bp.com/statisticalreview. (Accessed: 19th Sep 2018).

Cherng-Yuan Lin, Lin Yi-Wei, 2012. Fuel characteristics of biodiesel produced from high-acid oil from soybean soap stock by supercritical-methanol transesterification. Energies 5, 2370–2380. https://doi.org/10.3390/en5072370.

Chevalier, P.D., Proulx, D., Lessard, P., Vincent, W.F., Noue, J., 2000. Nitrogen and phosphorus removal by high latitude mat-forming cyanobacteria for potential use in tertiary wastewater treatment. J. Appl. Phycol. 12, 105–112. https://doi.org/10.1128/AEM.05275-11.

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25, 294–306. https://doi.org/10.1016/j.biotechadv.2007.02.001.

De Philippis, R., Paperi, R., Sili, C., 2007. Heavy metal sorption by released polysaccharides and whole cultures of two exo polysaccharide-producing cyanobacteria. J. Appl. Phycol. 18, 181–187.

Fagodiya, R.K., Pathak, H., Kumar, A., Bhatia, A., Jain, N., 2017. Global temperature potential of nitrogen use in agriculture: a 50-year assessment. Scientific Report Nature 7, 44928. https://doi.org/10.1038/srep44928.

Fayza, A.F., Abd-El Fattah, Z., 2008. Effect of Chlorella vulgaris as bio-fertiliser on growth parameters and metabolic aspects of lettuce plant. J. Agric. Soc. Sci. 4, 165–169.

Fertilizer Association of India FAI, 2015-2016. Fertilizer Statistics. www.faidelhi.org/virtual-library.htm. (Accessed 19 September 2018).

Food and Agriculture Organization of the United Nations, 2018. Rome, Italy. www.faostat.fao.org/(Accessed: 19th Sep 2018).

Government of India, Ministry Of Environment, 2018. Forest and Climate Change Lok Sabha Un-starred Question No.2541, 28th May.

Han, S.F., Jin, W.B., Tu, R.J., Wu, W.M., 2015. Biofuel production from microalgae as feedstock: current status and potential. Crit. Rev. Biotechnol. 35 (2), 255–268. https://doi.org/10.3109/07388551.2013.835301.

Ji, L., Xie, S., Feng, J., Li, Y., Chen, L., 2012. Heavy metal uptake capacities by the common fresh water green alga Cladophora fracta. J. Appl. Phycol. 24, 979–983.

Kabdasli, I., Tunay, O., Cetin, M.S., Olmez, T., 2002. Assessment of magnesium ammonium phosphate precipitation for the treatment of leather tanning industry wastewaters. Water Sci. Technol. 46, 231–239.

Khan, S.A., Malla, F.A., Rashmi, Malav, L.C., Gupta, N., Kumar, A., 2018. Potential of wastewater treating Chlorella minutissima for methane enrichment and CO2 sequestration of biogas and producing lipids. Energy 150, 153–163. https://doi.org/10.1016/j.energy.2018.02.126.

Khan, S.A., Rashmi, Hussain, M.Z., Prasad, S., Banerjee, U.C., 2009. Prospects of biodiesel production from microalgae in India. Renew. Sustain. Energy Rev. 13, 2361–2372. https://doi.org/10.3390/en5082667.

Kodandoor, S., Madaiah, R., 2011. Total lipid and fatty acid composition in some freshwater cyanobacteria. J. Algal Biomass Utln. 2, 83–97.

Lepage, G., Roy, C.C., 1984. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. J. Lipid Res. 25, 1391–1396.

Li, X., Hu, H.Y., Gan, K., Yang, J., 2010. Growth and nutrient removal properties of a freshwater microalga Scenedesmus sp. LX1 under different kinds of nitrogen sources. Ecol. Eng. 36, 379–381.

Malla, F.A., Khan, S.A., Rashmi, Sharma, G.K., Gupta, N., Abraham, G., 2015. Phycoremediation potential of Chlorella minutissima on primary and tertiary treated wastewater for nutrient removal and biodiesel production. Ecol. Eng. 75, 343–349. https://doi.org/10.1016/j.ecoleng.2014.11.038.

Martinez, M.E., Sanchez, S., Jimenez, J.M., Yousfi, F., Munoz, L., 2000. Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus. Bioresour. Technol. 73, 263–272. https://doi.org/10.1016/j.btre.2016.04.003.

Moore, R.D., Richards, G., Story, A., 2008. Electrical conductivity as an indicator of water chemistry and hydrologic process. Watershed Manag. Bull. 11 (2), 25–29.

Mulbry, W., Kondrad, S., Pizarro, C., 2007. Biofertilizers from algal treatment of dairy and swine manure effluents: characterization of algal biomass as a slow release fertilizer. J. Veg. Sci. 12 (4), 107–125. https://doi.org/10.1300/J484v12n04_08.

Mulbry, W., Westhead, E.A., Pizarro, C., Sikora, L., 2005. Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. Bioresour. Technol. 96, 451–458. https://doi.org/10.1016/j.biortech.2004.05.026.

Nathan, D.M., Meng, W., Sarina, J., Ergas, J.R., Mihelcic, A., Mulder, J., van de Vossenberg', J.B., van Lier' Peter, V.D.S., 2016. Biological nitrogen removal in a photosequencing batch reactor with an algal-nitrifying bacterial consortium and anammox granules. Environ. Sci. Technol. Lett. 3, 175.

Nunez, V., Voltolina, D., Nieves, M., Pina, M.A., Guerrero, M., 2001. Nitrogen budget in Scenedesmus obliquus cultures with artificial wastewater. Bioresour. Technol. 78, 161–164.

Pathak, H., Jain, N., Bhatia, A., Kumar, A., Chatterjee, D., 2016. Improved Nitrogen Management: a key to climate change adaptation and mitigation. Ind. J. fertil. 12 (11), 151–162.

Perez-Garcia, O., Escalante, F.M.E., de-Bashan, L.E., Bashan, Y., 2011. Heterotrophic cultures of microalgae: metabolism and potential products. Water Res. 45 (1), 11–36. https://doi.org/10.1016/j.watres.2010.08.037.

Prabakaran, P., Ravindran, A.D., 2011. Scenedesmus as a potential source of biodiesel among selected microalgae. Curr. Sci. 102 (4), 616–620. www.jstor.org/stable/24084122.

Rawat, I., Ranjith, R.K., Mutanda, T., Bux, F., 2011. Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Appl. Energy 3411–3424. https://doi.org/10.1016/j.apenergy.2010.11.025.

Ruiz-Marin, A., Mendoza-Espinosa, L.G., Stephenson, T., 2010. Growth and nutrient removal in free and immobilised green algae in batch and semi-continuous cultures treating real wastewater. Bioresour. Technol. 101, 58–64. https://doi.org/10.1016/j.biortech.2009.02.076.

Sengar, R.M.S., Singh, K.K., Singh, S., 2011. Application of phycoremediation technology in the treatment of sewage waste water to reduce pollution load. Indian J. Sci. Res. 2 (4), 33–39.

Sheehan, J., Dunahay, T., Benemann, J., Roessler, P., 1998. A Look Back at the U.S. Department of Energy's Aquatic Species Program - Biodiesel from Algae, NERL/TP-580–24190. National Renewable Energy Laboratory, Golden, CO, p. 80401.

Singh, J., Rawat, K.S., Kumar, A., Singh, A., 2013. Effect of sewage sludge and bio-fertilizers on physicochemical properties of alluvial soil. Biochem. Cell. Arch. 13 (2), 191–202.

Smil, V., 2016. Energy Transitions: global and National Perspectives. BP Statistical Review of World Energy. www.bp.com/statisticalreview. (Accessed 19 September 2018).

Stein, J. (Ed.), 1973. Handbook of Phycological Methods. Culture Methods and Growth Measurements, 448. Cambridge University Press. https://doi.org/10.1002/jobm.19750150322.

Sturm, B.S.M., Lamer, S.L., 2011. An energy evaluation of coupling nutrient removal

from wastewater with algal biomass production. Appl. Energy 3499–3506.

Su, Y., Mennerich, A., Urban, B., 2011. Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture. Water Res. 45, 3351–3358. https://doi.org/10.1016/j.watres.2011.03.046.

Su, Y., Mennerich, B.A., Urban, B., 2012. Comparison of nutrient removal capacity and biomass settle ability of four high-potential microalgal species. Bioresour. Technol. 124, 157–162. https://doi.org/10.1016/j.biortech.2012.08.037.

Tam, N.F.Y., Wong, Y.S., 2000. Wastewater nutrient removal by Chlorella pyrenoidosa and Scenedesmus sp. Environ. Pollut. 58 (1), 19–34. https://doi.org/10.1016/0269-7491(89)90234-0.

Thompson, R.B., Meisinger, J.J., 2002. Management factors affecting ammonia volatilization from l and-applied cattle slurry in the Mid Atlantic USA. J. Environ. Qual. 31, 1329–1338.

Volensky, B., 2001. Detoxification of metal-bearing effluents: biosorption for the next century. Hydrometallurgy 59, 203–216.

Wilkie, A.C., Mulbry, W.W., 2002. Recovery of dairy manure nutrients by benthic freshwater algae. Bioresour. Technol. 84, 81–91.

World Water Development Report (WWDR), 2017. Wastewater: the Untapped Resource. UNESCO. WWAP (United Nations World Water Assessment Programme). Paris. (Accessed 19 September 2018).

Yu, K.L., Show, P.L., Ong, H.C., Ling, T.C., Chi-Wei Lan, J., Chen, W.H., Chang, J.S., 2018. Microalgae from wastewater treatment to biochar - feedstock preparation and conversion technologies. Energy Convers. Manag. 164, 188–197.

Zhang, E.D., Wang, B., Wang, Q.H., Zhang, S.B., Zhao, B.D., 2008. Ammonia-nitrogen and orthophosphate removal by immobilised Scenedesmus spp isolated from municipal wastewater for potential use in tertiary treatment. Bioresour. Technol. 99, 3787–3793.

Zhang, Y., Safa, N.J., Angelidaki, I., 2011. Simultaneous organic carbon, nutrients removal and energy production in a photo microbial fuel cell (PFC). Energy Environ. Sci. 4, 4340–4346.

Zhou, C., Lai, C., Xu, P., Zeng, G., Huang, D., Zhang, C., Cheng, J., Hu, M., Wan, L., Liu, Y., Xiong, W., Deng, Y., Wen, M., 2018a. In situ grown AgI/Bi12O17Cl2 hetero junction photocatalysts for visible light degradation of sulfa methazine: efficiency, pathway, and mechanism. ACS Sustain. Chem. Eng. 6 (3), 4174–4184.

Zhou, C., Lai, C., Xu, P., Zeng, G., Huang, D., Zhang, C., Cheng, J., Cheng, M., Hu, L., Wan, J., Chen, F., Xiong, W., Deng, R., 2018b. Rational design of carbon-doped carbon nitride/Bi12O17Cl2 composites: a promising candidate photocatalyst for boosting visible-light-driven photocatalytic degradation of tetracycline. ACS Sustain. Chem. Eng. 6 (5), 6941–6949.

Glossary

MoEF&CC, GOI: Ministry of Environment and Forest and Climate Change, Government of India
MTOE: Million Tonnes of Oil Equivalent
TWh: Terawatt-hours
RPM: Rounds per minute
WWDR: World Water Development Report
MLD: Millions of Liters per Day
IARI: Indian Agricultural Research Institute
GHGs: Green House Gases
NPK: Nitrogen Phosphorus Potassium